THE LIFE HISTORY OF EPIBDELLA MELLENI MACCALLUM 1927, A MONOGENETIC TREMATODE PARASITIC ON MARINE FISHES

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At present our knowledge concerning the life histories of the members of the order Monogena is extremely scanty. Although the life histories of the monogenetic trematodes are apparently much simpler than those of the digenetic forms, they have been less extensively studied, and definite information concerning them is, with a few exceptions, fragmentary. Of the sub-order Monopisthodiscinea Fuhrmann the only life history known is that of *Gyrodactylus* (Kathariner, 1904). Of the sub-order Monopisthocotylinea Odhner, which includes *Epibdella*, no life histories have been reported. In the sub-order Polyopisthocotylinea Odhner two life histories, those of *Polystomum integerrimum* (Zeller, 1872, 1876) and *Diplozoön paradoxum* (Zeller, 1872*a*), have been reported. The dimorphic development of *Polystomum integerrimum* on the gills of the tadpole and in the urinary bladder of the frog as given by Zeller (1872) has been questioned by several workers, including Stunkard (1917) who states that "the findings of Zeller are so unusual that one is led strongly to suspect he confused two different species." This leaves our definite knowledge of monogenetic life histories limited to two genera, *Gyrodactylus* and *Diplozoön*. The present investigation of *Epibdella melleni* MacCallum 1927 presents the third definitely-known life history of the Monogena and the first of the sub-order Monopisthocotylinea.

The adult of *Epibdella melleni* was first described by MacCallum (1927) as parasitic on the Pacific puffer (*Spheroides annulatus*), the spadefish (*Chaetodipterus faber*), and various species of angel fishes (*Angelicthys* and *Pomacanthus*) from the New York Aquarium. Due to the necessarily closed salt water system of the Aquarium, the infection spread rapidly to all tanks which contained susceptible fishes, and for several years it has been a very grave source of danger to all susceptible fishes.

This study was undertaken at the suggestion of Mr. Charles M. Breder of the New York Aquarium and Professor H. W. Stunkard of

New York University. The writers wish to thank Mr. Breder for his constant aid in the collection of material, for the identification of the fishes, and for information regarding their susceptibility, and Professor Stunkard for his helpful suggestions during the preparation of the manuscript. This study was made possible by a grant of the New York Zoölogical Society in the form of a Research Fellowship which was held by the senior author during the summer of 1930 and by the junior author during the academic year 1930–31.

Material and Methods

All material used for this study was obtained from infected fishes at the New York Aquarium. Adult specimens were obtained by scraping the body and cornea of the fish with a scalpel. In this way considerable mucus was obtained along with the parasites. This material was placed in small stender dishes, covered with sea water, and allowed to stand for ten minutes or longer. It was found that the parasites became firmly attached to the bottom of the dish, and that the mucus material could easily be removed with a pipette. After several changes of sea water the organisms, attached to the dish, could be studied with the aid of a binocular. In this way observations of the process of egg-laying were possible. It was found that the organisms remained attached to the dish after fixation. Therefore they were fixed, stained, destained, and cleared while attached by changing the fluid in the stender dish. They were then removed to a slide with the aid of a pipette and covered with balsam. Schaudinn's fluid and saturated aqueous solution of mercuric chloride plus five per cent acetic acid were used as fixatives. Whole mounts were stained with paracarmine, and sections with Delafield's hæmatoxylin and erythrosin.

Larvæ were obtained by collecting eggs and allowing them to hatch. Whenever larvæ were wanted, adult worms were collected and allowed to remain in stender dishes. The worms rarely lived more than twenty-four hours, but during this time numerous eggs were usually laid. These were removed to another dish in which the water was changed several times a day. At the end of five to eight days the eggs hatched, and the larvæ could be isolated with the aid of a mouth-controlled pipette.

Morphology of the Adult

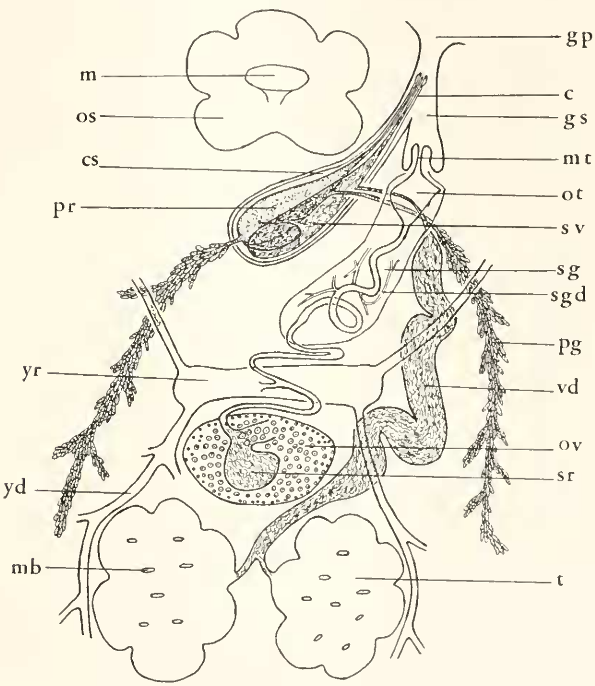
The adult of *Epibdella melleni* was described in some detail by MacCallum (1927), and this original description is substantiated by most of the observations of the present authors. The principal differences between the description as given by MacCallum and the present material lies in the details of the reproductive system and in the presence of fourteen larval or accessory hooks in the posterior sucker.

The adult of *E. melleni* (Fig. 1) varies from 3.5 to 5 mm. in length. The worms are usually white but sometimes contain numerous small patches of yellow pigment, and the covering of the body is smooth and unarmed. Two pairs of eyes are present near the anterior end of the body. The adhesive organs consist of one large posterior sucker and two smaller anterior suckers, all located on the ventral side of the body. The posterior sucker is round, smooth, and very shallow. It is 1.2 mm. in diameter in 4.5 mm. specimens and bears three pairs of large spines but no papillæ. The most anterior pair of spines is about .22 mm. in length, is slightly forked at the base, and is directed anteriorly, while the other two pairs, more posterior in position, arise posteriorly but are recurved in such a manner as to form veritable hooks whose points are directed anteriorly. The middle pair is the largest and most powerful, measuring about .30 mm. in length; the most posterior pair is much smaller, being only about .12 mm. in length, and its recurved points are relatively fine. There are also fourteen very small (10 μ in length) larval or accessory hooks arranged radially close to the margin of the sucker. These hooks are actually present on the larva and are apparently homologous with the larval hooklets of other monogenetic trematodes. Each of these hooklets is branched, and the two points are opposed (Fig. 13). The margin of the posterior sucker bears a secondary ring of very thin muscular tissue which serves to make the closure of the sucker on the substratum more secure and certain (Fig. 3). The anterior suckers are unarmed and bear no extra peripheral ring of muscular tissue. Both arcuate and dorso-ventral muscle fibers may be seen in sections of the suckers, but the greater portion of the suckers is made up of dorso-ventral fibers.

The oral sucker is large and powerful and is located in the median plane behind the anterior pair. The sucker is almost completely covered by thin lip-like structures which are a continuation of the dermal and epithelial layers of the ventral body wall (Fig. 2). These form a flat pouch whose opening coincides with that of the mouth sucker. Apparently these lips must be retracted when the sucker is attached to the substratum. When closed the oral sucker is pentalobate as shown in Fig. 1. It opens directly into the short œsophagus; there is no muscular pharynx. From the œsophagus arise the two main lateral intestinal trunks which have branches from practically all parts of the body. Each lateral intestinal trunk receives about fourteen smaller branches, and each of these smaller branches is further subdivided. No anastomosis of the branches could be definitely traced in sections, but MacCallum (1927) has described anastomoses of the branches in the median posterior region of the body. However, since anastomosis of the digestive cæca is quite common in other genera of the Monogena, it is possible that it occurs in this species.

The nervous system is composed of a crescent-shaped ganglionic mass anterior to the oral sucker, and two pairs of longitudinal nerve trunks, one of which lies just ventral to the main excretory channels, and the other of which lies more laterad (Fig. 1). The four eyes are embedded in the anterior ganglionic mass. The two trunks on each side are united to each other by three cross-connections, and they fuse in the posterior region of the body so that only two instead of four trunks enter the posterior sucker. In the posterior sucker these two trunks turn mediad and unite, forming a virtual 'ring' system, composed of two pairs of longitudinal trunks united anteriorly by two ganglionic masses and posteriorly by a connection in the posterior sucker. Anteriorly from the anterior ganglionic mass there arise two short trunks which innervate the anterior suckers, and these trunks also are united by a cross-connection. The smaller nerves branch off from the longitudinal trunks and from the cross-connection in the posterior sucker, which seems to serve as a nerve plexus for the numerous small branches which innervate the sucker. In some sections it appeared as if there were another cross-connection, semicircular in shape, in the posterior sucker besides the one shown in the figure, but this could not be traced with certainty.

The excretory pores are two in number and open to the dorsal side of the body, laterad to the posterior margin of the mouth. The two main excretory channels lead posteriorly from the excretory pores and unite near the posterior margin of the body. Slightly posterior to the excretory pores these channels widen to form the excretory vesicles which are very noticeable in the living adult. The longitudinal channels receive many branches, and these branches are repeatedly bifurcated in such a manner that the terminal ends ramify to all regions of the posterior two-thirds of the organism. Anteriorly from each excretory vesicle arises another longitudinal channel which passes forward, is greatly bifurcated, and curves mediad between the oral and anterior suckers. These two anterior channels unite in the median line behind the eyes so that the four main channels form virtually a 'ring' system composed of longitudinal channels united close to the anterior and posterior ends of the worm. The main channels in the body and also in the posterior sucker are identical in position and in connections with those of the larva which will be described later. The excretory system of the adult is obviously an elaboration of the pattern present in the larva, but the details of the system of the adult have not been traced. It is considered doubtful, however, whether there are four main longitudinal channels posterior to the excretory vesicles as described by MacCallum (1927), and it is



TEXT FIG. 1. Semi-diagrammatic drawing of reproductive system of *Epibdella melleni.* Abbreviations: *c*, cirrus; *cs*, cirrus sac; *gs*, genital sinus; *m*, mouth; *mb*, muscular band through the testis; *mt*, metraterm; *od*, oviduct; *os*, oral sucker; *ot*, oötype; *ov*, ovary; *pg*, prostate gland; *pr*, prostatic reservoir; *sg*, 'shell gland'; *sgd*, 'shell gland' duct; *sr*, seminal receptacle; *sv*, seminal vesicle; *t*, testis, *vd*, vas deferens; *yd*, vitelline duct; *yr*, yolk reservoir.

thought probable that in his description the nervous system has been confused with the excretory.

The male reproductive system (text figure 1) is rather unusual in several respects. There are two testes (approximately .4 by .5 mm. in 4.5 mm. specimens) which lie in the mid-portion of the body; they are almost round in general outline with a lobate margin as seen from the dorsal or ventral surfaces, the amount of lobulation apparently increasing with the size of the specimen. They are not smooth bodies as described by MacCallum (1927). The testes of adult specimens are pierced by two to ten bands of muscle tissue which extend through the organs in a dorso-ventral direction. Their presence indicates that embryologically the testes might have been formed by the fusion of numerous small testicular bodies. Apparently the contraction of the muscles would force the contents of the testes into the short vasa efferentia which unite to form one very large sinuous vas deferens which passes forward on the left of the ovary. At the level of the expanded portion of the oötype the vas deferens is constricted and turns dorsad and mediad to enter the seminal vesicle. The ejaculatory apparatus consists of a very large pear-shaped cirrus sac located approximately in the median line directly posterior to the mouth; the smaller end of the organ tapers to open into the genital sinus to the left of the mouth. The cirrus sac is divided by a partition into two pyriform sacs: one is the seminal vesicle which receives spermatozoa from the vas deferens entering at the anterior end of the bulbous portion; the other is the prostatic reservoir which receives the prostate secretion. The prostate consists of two long, narrow, slightly branching, acinous glands; the duct of one enters the cirrus sac at its anterior end, and of the other at the anterior end of the bulbous portion at the same level as does the vas deferens. The posterior ends of the seminal vesicle and of the prostatic reservoir are folded back upon themselves in some of the specimens examined. The cirrus is a long, slender, heavy-walled tube continuous with the wall of the cirrus sac. It is divided internally by a septum continuous with its outer wall, thus forming two separate channels which extend to the end of the organ. One of these channels is continuous with the seminal vesicle and the other with the prostatic reservoir. The prostatic secretion stains deeply with erythrosin, and in sections or whole mounts gives a deep red color to the prostate glands, to their ducts, and to the prostatic reservoir. The secretion appears granular in stained sections and hyaline in living specimens. The cirrus is unarmed, but the end is marked off by a slight stricture. The cirrus sac opens into the genital sinus which is divided by a septum into two portions, a medial which receives the cirrus and a lateral into which the metraterm opens.

The female reproductive system of *E. melleni* (text figure 1) is quite simple in outline but has an unusual feature in the position of the seminal receptacle. The ovary lies in the mid-portion of the body, in the median plane, just anterior to the testes. It is oval and measures about .30 by .22 mm. in 4.5 mm. specimens. The seminal receptacle is definitely within the ovary and can be demonstrated as such in cross, sagittal and frontal sections, thus obviating the possibility that it is above or below the ovary. It may be clearly seen to contain spermatozoa when observed in sections with an oil immersion lens. The oviduct begins near the center of the ovary and is connected close to its origin with the seminal receptacle. Since both spermatozoa and ripe eggs are found in the initial portion of the oviduct and in the anterior part of the seminal receptacle, it is believed that fertilization takes place in this region. The oviduct passes out of the ventro-anterior portion of the ovary and arches dorsad and to the left. It then turns ventrad and crosses under the yolk reservoir from which it receives the yolk duct. The oviduct then continues tortuously to the oötype. The oötype is a long, slender, very muscular, coiled structure with a greatly enlarged tetrahedral anterior end. A uterus is absent, and the oötype opens via the metraterm into the lateral division of the genital sinus. The oötype is surrounded by a rather extensive 'shell gland' embedded in loose connective tissue. The ducts from the 'shell gland' unite to form several common tubules which open into the posterior portion of the oötype. A vagina and genito-intestinal canal are absent, and the vitellaria fill most of the interstitial space between the other organs throughout the body of the worm.

The Process of Egg Laying

In the living adult the yolk reservoir and the vitelline ducts may easily be distinguished with the aid of a low-powered binocular microscope, especially when they are full of yolk cells which appear as large gray structures against the clear body of the organism. When an egg is to be formed, a seemingly large quantity of yolk material streams from the yolk reservoir into the oötype, and it is driven back and forth by a rapid reversible peristaltic movement of this structure. The greater mass of yolk comes to lie in the anterior tetrahedral end of the oötype. During this time the shell material must lie around the yolk, and it is visible as a hyaline covering of the filament of the egg. The filament is at first filled with a central core of yolk material. This yolk material, due to peristaltic movements of the surrounding oötype, is driven forward into the tetrahedral region of the oötype, and further contraction of the posterior region of the oötype results in the shaping of the filament which consists at this stage of only hyaline material. During this time the yolk material at the anterior end of the egg is being shaped into its characteristic tetrahedral form. As the formation of the filament is completed the shell material may be distinguished around the body of the egg. The surging movement of the yolk continues until the egg is completely shaped, and then the shell material begins to harden. As it hardens it becomes yellowish, and this color gradually becomes deeper. When formation of the egg is complete, the anterior suckers, if attached, release their hold on the substratum, and the anterior end of the organism curves dorsad. The egg is then very quickly shot out from the anteriorly directed genital pore. The filament sometimes remains caught in the genital opening, but more often the egg is thrown completely free of the mother organism. After elimination of the egg, the shell material becomes a very deep yellow-bronze and is quite opaque.

Usually within a few seconds after the discharge of an egg, more yolk material flows from the yolk reservoir into the oötype, and the formation of another egg is begun. The whole process of egg formation requires from two to ten minutes, and the average time is about five minutes. The time-controlling factor seems to be the rapidity with which yolk material may be supplied to the yolk reservoir. Sometimes the yolk reservoir becomes completely emptied, and the process of egg-laying is stopped for a few minutes until the reservoir is refilled from the vitelline ducts. In some few organisms which seemed to lack sufficient shell material, the eggs were incompletely formed. In such cases the shell material usually formed the filament and the posterior portion of the tetrahedron, and the yolk material was shot out in a stream and was followed by the filament at the time the egg would have been released had it been complete (Fig. 12).

The Egg

The egg of *Epibdella melleni* is tetrahedral in form and usually bears a single long filament and two shorter filaments with curved ends which form veritable hooks (Fig. 8). However, a number of eggs have been observed without hooks (Fig. 9), and others have been seen with three hooks and no filament. All of these types of eggs have been observed to come from the same adult, thus eliminating the possibility that they were eggs of different species. The size of the body of the egg is somewhat variable, but usually measures about .13 mm. on the edges of the tetrahedron. The filament is from .8 to 1.2 mm. in length and is sometimes seen to contain small ellipsoidal cavities throughout part of its length. The length of the filament, the presence or absence of and length of hooks, and the number of cavities in the long filament seem to be determined by the amount and distribution of shell material in the oötype at the time the egg is shaped. In some instances slightly larger and somewhat globiform eggs are produced (Fig. 11). These seem to result from a lack of sufficient shell material or from the presence of too much yolk in the oötype at the time of egg formation. The normal eggs are yellow in color when formed but turn to a golden bronze shortly after they are laid.

Hatching of the Egg, and Behavior of the Larva

Eggs of *Epibdella melleni*, isolated into small stender dishes in which the sea water is changed daily, hatch at room temperature in five to eight days. On the fourth to sixth days the larvæ may be seen within the eggs, and if an egg is broken open the larva is found apparently completely formed but non-motile. Just before hatching, the larva may be seen squirming about within the shell. A small circular opening is formed in the rounded corner of the egg, that is, on the corner which bears neither filament nor hook, but a preformed operculum was not observed. The larva emerges anterior end first. The anterior ciliated portion is thrust out, and the cilia meanwhile beat very rapidly. The larva may remain in this position for fifteen minutes. Then the beating of the cilia slowly pulls the organism out until only the posterior sucker remains within the shell. At the end of another fifteen minutes the larva is usually completely emerged. However, the time required for hatching is presumably affected by numerous undetermined factors. The larva, after emergence, swims very rapidly through the water, pausing only momentarily now and then on the bottom of the dish or on solid objects in the water. Within six hours, if the larva does not become attached to a suitable host, it apparently becomes exhausted, the rate of movement decreases, and the organism finally settles to the bottom, capable of only a slow crawling movement. This is presumably due to a loss of the ciliated epithelium, since such larvæ are always almost completely deciliated. Up to this time the posterior sucker does not appear to be functional.

Morphology of the Larva

The ciliated larva of *Epibdella melleni* (Fig. 4) is approximately 225 microns in length and 60 microns in width. It is slightly flattened dorso-ventrally in the anterior region, and, otherwise, is fusiform in general shape except for a constriction in the region of the mouth. The posterior third of the larva is composed of the posterior sucker which, at the time of hatching and for some time afterward, remains folded in such a manner as to be non-functional as a sucker. The anterior two-thirds of the larva is composed of what becomes the body of the adult, and it is, in general structure, similar to that of the adult except that the digestive and excretory systems are simple and the reproductive systems are not yet developed. The oral sucker is round and muscular and is located in the anterior part of the middle third of the body proper. It opens into a very short œsophagus which leads into two relatively large digestive cæca. These continue latero-posteriorly almost to the end of the body. No diverticula are present. Anterior to the mouth on the dorsal surface are two pairs of eyes. These are cup-shaped masses of pigment from the cavity of which protrude spherical hyaline lenses. Each of the posterior pair of eyes is approximately 16 microns in diameter and is directed anterio-laterad. Those of anterior pair are 12 microns in diameter and are directed posterio-laterad. The anterior suckers are not distinct sucking disks but are pad-like muscular areas, easily distinguishable in the living form.

The excretory system is composed of two relatively large excretory vesicles which are located slightly posterior and laterad to the mouth, four ducts which lead from them, and ten pairs of flame cells. The vesicles are quite distinct in the living organism and appear as large vacuolar structures. The excretory system opens by dorsal pores located on either side of the mouth as in the adult. The excretory system is shown diagrammatically in Fig. 7. Two large excretory channels extend, one from each vesicle, posteriorly into the posterior sucker. They are joined with each other by a cross-channel at the posterior end of the body proper. Each of these channels gives off an anteriorly directed branch which ends in a single flame cell about halfway between the vesicle and the cross-channel. The longitudinal channels continue into the sucker where each branches and ends in five flame cells as shown in Fig. 7. Leading anteriorly from the excretory vesicles is another pair of channels, one from each vesicle. These unite anterior to the mouth to form one median channel which continues forward between the eyes and branches laterally, and then each branch bifurcates anterio-posteriorly and ends in flame cells. In the region of the mouth are four flame cells, one on each side of the anterio-lateral and posterio-lateral sides of the oral sucker. The channels of these cells are probably branches of the anterior longitudinal channels, but this could not be determined with certainty due to the thickness of the oral sucker and the proximity of the ducts to it. These supposed connections are shown in Fig. 7. The arrangement of the excretory ducts of the larva is virtually a 'ring' system with two lateral excretory pores. The longitudinal channels and cross-connections of the larva have been checked in older specimens and have been found to be the main excretory channels of the adult.

The organism bears cilia in the anterior, middle and posterior regions. The anterior ciliated region extends from the anterior pair of eyes forward, and cilia cover practically all of the anterior region except the sucking pads. The middle ciliated region, extending from the posterior edge of the excretory vesicles almost to the posterior end of the body proper, is covered with cilia on the lateral, latero-dorsal, and latero-ventral surfaces. No cilia were seen on the mid-dorsal and mid-ventral surfaces in this region. The posterior ciliated region includes the lateral and dorsal surfaces of the posterior two-thirds of the posterior sucker. The cilia are relatively long and arise from an epithelial layer which can be seen distinctly in living specimens. This epithelial layer contains many large highly refractile granules of unknown function. When cilia are lost, the epithelial layer from which they arise is shed with them.

The posterior sucker, as folded when the larva is free-swimming, contains the definitive spines characteristic of the adult. These lie in a longitudinal position with the curved ends directed mediad as seen from the ventral surface. At the margin of the folded sucker, and lying ventrad to the definitive spines, may be seen the accessory or larval hooks. These are all the same size and measure approximately 10 microns in length. A single hooklet is shown in Fig. 13.

When maintained under a sealed coverslip, the ciliated larva may be seen to lose its cilia and to assume a shape and position more characteristic of the adult. The anterior sucking pads are usually very active in fresh preparations; these are stretched forward and are attached to the slide, and the body may be drawn afterward. The organism is capable of moving about in this fashion as well as by the use of the cilia. After this type of movement has been continued for some time, the ciliated epithelium begins to slough off, leaving areas of the normally ciliated region devoid of cilia. Apparently the first regions to become deciliated are those in the vicinity of the anterior sucking pads and those which cover the posterior sucker. However, the other ciliated epithelium is shed shortly afterward. If free-swimming larvæ are selected at random and examined, many are found which do not bear their full quota of cilia due to the sloughing of the epithelium. This may give rise to considerable confusion concerning the normal distribution, and it was necessary to examine a number of specimens in order to obtain the distribution shown in Fig. 4. Concomitantly with the sloughing of the ciliated epithelium, the posterior sucker is unfolded. As it is spread out, the definitive spines turn upon their longitudinal axis so that the curved ends are directed laterad, and the accessory hooks are pointed radially around the margin of the sucker. At this time the posterior sucker is functional and becomes firmly attached to the slide. Such an organism is shown in Fig. 5. This drawing was made of a larva which was isolated when ciliated and which had undergone the above transformations in about forty-five minutes while under observation.

In an attempt to interpret these transformations in relation to the normal life history of the organism, it is assumed that the larva is free-swimming until it comes in contact with a susceptible fish. Attachment is first by means of the anterior sucking pads. Then the cilia are lost, and the posterior sucker is unfolded, and a firmer attachment is afforded by means of the fourteen accessory hooks of the posterior sucker, aided perhaps by the most posterior pair of definitive spines.

Development of the Attached Form

After attachment the first morphological change to be noticed is the development of the anterior sucking pads into definite suckers. Then the mouth, which is round in the ciliated form, becomes lobate in outline as is that of the adult, and the digestive cæca show signs of becoming diverticulated. The posterior sucker becomes slightly larger in proportion to the size of the body, and the large spines increase in size and change in shape. A specimen in this stage that was obtained from an infested fish is shown in Fig. 6. No increase in size of the larval or accessory hooks could be noted, even when measurements of these hooks in young larvæ and in adult organisms were compared. All accessory hooks measured were between 9.8 μ and 10.7 μ in length. For this reason it is believed that these hooks are not of especial importance in the adult.

Further development of the organism seems to involve principally a further bifurcation of the digestive cæca, an elaboration of the excretory pattern, and the development of the reproductive system. The relatively large amount of space occupied by the reproductive system in the adult is responsible for the great differences in general appearance of the young (Fig. 6) and of the adult forms.

The sizes of the eyes change very little during the growth of the organism. Measurements made of the eyes of newly attached larvæ and of adult specimens showed no significant difference in size. The more anterior pair of eyes was found to be 10–13 μ in diameter, and the posterior pair was 15–18 μ in both young and adult specimens. However, the posterior pair of eyes in the adult is directed anterio-mediad instead of anterio-laterad as in the larva. The eyes of the free-swimming larvæ are quite symmetrical in outline as shown in Fig. 4. Those of the adults are usually not spherical but are flattened in one direction or another. The eyes of some adults even appear conical with the base of the cone adjacent to the lens.

During the growth of the individual the relative amounts of growth of the three pairs of spines are not equal. The middle and the more anterior pairs grow at a rate that is relatively about twice that of the most posterior pair. Thus, the sizes of the first two pairs in the adult are over six times that of the same pairs in the youngest attached form, while the size of the most posterior pair is only three times that of the young form. Table I shows the average measurements of the three pairs of spines in young, medium-sized (about 1.5 mm.), and adult (over 3.5 mm.) specimens, and also the ratio of the sizes of the spines in the various sized specimens, the youngest forms being used as unity. The figures are the averages of the measurements of six specimens, and all three pairs were measured on the same individual. Therefore the ratios are strictly comparable.

Occurrence and Pathogenicity

When the adult of *Epibdella melleni*, obtained from the tanks of the New York Aquarium, was first described by MacCallum (1927),

Table I

	Average size of spines in mm.			Ratio of the size of the spine to that of the young attached form		
Pair of spines	Anterior	Middle	Posterior	Anterior	Middle	Posterior
Young.............	.035	.045	.038	1.0	1.0	1.0
Medium...........	.109	.174	.086	3.1	3.9	2.3
Adult.............	.216	.297	.118	6.2	6.6	3.0

it was stated that the infection was probably introduced by a Pacific puffer (*Spheroides annulatus*) from California. However, it is the belief of Mr. Breder and his associates that there was no Pacific puffer in the Aquarium for some time preceding the discovery of the parasite and that the parasite was originally introduced and is being continually reintroduced with shipments of fishes from Key West and Nassau. Furthermore, the parasite occurs in both the Chicago and Philadelphia Aquariums, neither of which has Pacific fishes. Also, it is known that *E. melleni* will not survive very long exposure to the acid water of New York harbor and that it will multiply rapidly in the neutral tank water of the Aquarium. For many years no attempt was made to control the chemical composition of the water in the tanks of the New York Aquarium, and only after the installation of an efficient means of chemical control did the parasites become numerous, probably due to the very high acidity of the water previous to that time. For these reasons it is believed that the parasite is a West

Indian species and that it might have been continually present in small numbers in the New York Aquarium for many years before its discovery in 1927.

The fishes which have been found to be susceptible to infection with *Epibdella melleni* are as follows:

Subclass Teleostomi
- Order Acanthopteri
  - Family Carangidæ
    - *Caranx crysos* (Mitchill), Runner (X)
    - *Caranx hippos* (Linnaeus), Common Jack (X)
    - *Naucrates ductor* Linnaeus, Pilot Fish
    - *Trachinotus carolinus* (Linnaeus), Common Pompano
    - *Trachinotus glaucus* (Bloch), Old Wife or Palometa
    - *Vomer setapinnis* (Mitchill), Moonfish
  - Family Pomatomidæ
    - *Pomatomus saltatrix* (Linnaeus), Bluefish
  - Family Serranidæ
    - *Centropristus striatus* (Linnaeus), Common Sea Bass (X)
    - *Dermatolepis punctatus* Gill, Spotted Grouper (Pacific) (X)
    - *Epinephelus adscensionis* (Osbeck), Rock Hind
    - *Epinephelus guttatus* (Linnaeus), Red Hind
    - *Epinephelus morio* (Cuvier and Valenciennes), Red Grouper (X)
    - *Epinephelus striatus* (Bloch), Nassau Grouper (X)
    - *Paralabrax maculatofasciatus* (Steindachner), Spotted Cabrilla (Pacific)
    - *Promicrops itaiara* (Lichtenstein), Jewfish
  - Family Lutianidæ
    - *Lutianus analis* (Cuvier and Valenciennes), Muttonfish
    - *Lutianus apodus* (Walbaum), Schoolmaster
    - *Lutianus jocu* (Bloch and Schneider), Dog Snapper
    - *Lutianus synagris* (Linnaeus), Spot Snapper (X)
  - Family Hæmulidæ
    - *Anisotremus surinamensis* (Bloch), Black Margate
    - *Anisotremus virginicus* (Linnaeus), Porkfish
    - *Hæmulon album* Cuvier and Valenciennes, Margate
  - Family Sciænidæ
    - *Menticirrhus saxatilis* (Bloch and Schneider), Kingfish
    - *Micropogon undulatus* (Linnaeus), Croaker
  - Family Labridæ
    - *Lachnolaimus maximus* (Walbaum), Hogfish
    - *Tautoga onitis* (Linnaeus), Tautog
  - Family Ephippidæ
    - *Chætodipterus faber* (Broussonet), Spadefish
  - Family Chætodontidæ
    - *Angelichthys ciliaris* (Linnaeus), Queen Angelfish
    - *Angelichthys isabelita* Jordan and Ritter, Blue Angelfish
    - *Angelichthys townsendi* Nichols and Mowbray, Townsend's Angelfish
    - *Chætodon ocellatus* Bloch, Common Butterfly Fish
    - *Pomacanthus arcuatus* (Linnaeus), Black Angelfish
    - *Pomacanthus paru* (Bloch), French Angelfish
  - Family Acanthuridæ
    - *Acanthurus cæruleus* Bloch and Schneider, Blue Tang
    - *Acanthurus hepatus* (Linnaeus), Brown Tang or Doctor Fish
  - Family Balistidæ
    - *Balistes vetula* Linnaeus, Queen Triggerfish
    - *Melichthys bispinosus* Gilbert, Pacific Black Trigger (X)

Family Monacanthidæ
Ceratacanthus schœpfii (Walbaum), Orange Filefish
Stephanolepis hispidus (Linnaeus), Common Filefish
Family Ostraciidæ
Lactophrys tricornis (Linnaeus), Cowfish
Lactophrys trigonus (Linnaeus), Common Trunkfish
Lactophrys triqueter (Linnaeus), Smooth Trunkfish
Family Tetradontidæ
Spheroides annulatus (Jenyns) Pacific Puffer (X)
Spheroides maculatus (Bloch and Schneider), Common Puffer or Northern Swellfish
Family Diodontidæ
Diodon hystrix Linnaeus, Porcupine Fish
Family Triglidæ
Prionotus evolans (Linnaeus), Striped Sea Robin
Family Malacanthidæ
Malacanthus plumeri (Bloch), Sandfish

The species marked "X" seems to have developed a partial immunity after a short period of susceptibility. Most of these species are usually present in the tanks of the Aquarium, and newly arrived specimens always show a marked susceptibility to infection. However, after being present in the tanks for several weeks these species seldom show a slight and never a serious infection although they are continually exposed to reinfection. Some of the other susceptible fishes (*e.g.*, *Chætodipterus faber*, the spadefish) seemingly retain their infections, continually become reinfected, and die if they do not receive treatment. The central members of the spiny-rayed fishes (Acanthopteri), especially members of the families Serranidæ and Lutianidæ, are extremely susceptible, and the possibility of the development of an immunity seems to be more strongly suggested in these families although it is not shown by all members. The other species showing a distinct susceptibility are rather scattered phylogenetically but are all within this order.

Epibdella melleni has never (prior to June 1931) been observed on any of the following fishes, all of which have been continually exposed to infection while at the Aquarium:

Subclass Elasmobranchii
Order Asterospondyli
Family Ginglymostomidæ
Ginglymostoma cirratum (Bonnaterre), Nurse Shark
Family Galeidæ
Mustelus canis (Mitchill), Smooth Dogfish
Family Carchariidæ
Carcharias littoralis (Mitchill), Sand Shark
Order Batoidei
Family Rajidæ
Raja eglanteria Lacépède, Clear-nosed Skate

Family Dasyatidæ
Dasyatis centrura (Mitchill), Northern Sting Ray
Family Aëtobatidæ
Rhinoptera quadriloba (Le Sueur), Cow-nosed Ray

Subclass Teleostomi
Order Apodes
Family Murænidæ
Gymnothorax funebris Ranzani, Green Moray
Gymnothorax moringa (Cuvier), Spotted Moray
Order Haplomi
Family Pœciliidæ
Fundulus heteroclitus (Linnaeus), Common Killifish
Fundulus majalis (Walbaum), Striped Killifish
Order Lophobranchii
Family Syngnathidæ
Hippocampus hudsonius De Kay, Northern Sea Horse
Order Acanthopteri
Family Serranidæ
Mycteroperca bonaci (Poey), Black Grouper
Mycteroperca microlepis (Goode and Bean), Gag
Roccus lineatus (Bloch), Striped Bass
Family Lutianidæ
Evoplites viridis (Valenciennes), Blue-striped Snapper (Pacific)
Lutianus griseus (Linnaeus), Gray Snapper
Family Hæmulidæ
Hæmulon sp., Grunts
Family Sparidæ
Archosargus pourtalesii (Steindachner), Pacific Salema
Archosargus probatocephalus (Walbaum), Sheepshead
Family Sciænidæ
Leiostomus xanthurus Lacépède, Spot
Pogonias cromis (Linnaeus), Black or Sea Drum
Family Pomacentridæ
Pomacentrus rectifrænum (Gill), Pacific Beau Gregory
Family Diodontidæ
Chilomycterus schœpfii (Walbaum), Spiny Boxfish
Family Echeneidæ
Echeneis naucrates Linnaeus, Shark Sucker
Family Batrachoididæ
Opsanus tau (Linnaeus), Toadfish

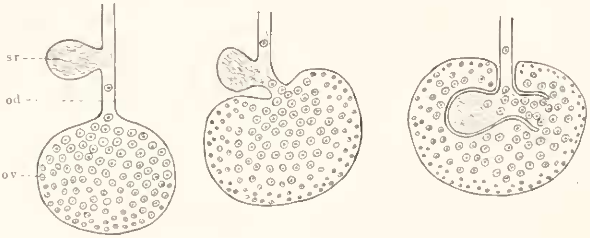
It is to be noted that although six elasmobranchs, representing six different families, were exposed, not one was ever observed to be infected. Also, five orders of the teleosts are represented on the list of non-susceptible fishes, and only one order, the Acanthopteri, is represented on the list of susceptible fishes. Therefore it seems as if all susceptible fishes may belong to the order Acanthopteri. Of this order a high susceptibility is shown by members of the families Serranidæ, Lutianidæ, and Ephippidæ, and no infections have been observed in the families Sparidæ, Pomocentridæ, Echeneidæ, and Batrachoididæ. Some of the other families are represented by members on both the susceptible and supposedly non-susceptible lists, and others are represented on the susceptible list only.

The injury produced to a susceptible fish is usually quite considerable, and if the infection is not treated, death often results. The trematodes attach themselves to the epidermis and to the conjunctiva, and in some cases they have been found in the gill and nasal cavities. Young specimens may be found on almost any part of the epidermis, but in some species (*e.g.*, *Chætodipterus faber* and *Promicrops itaiara*) the adults seem to be concentrated on the eyes. This concentration may be brought about either by the migration of young forms to the eyes, or by a high mortality rate of those which have become attached to the epidermis, or by both of these possible factors. The relative ease with which nourishment could be obtained from the soft tissue of the conjuctiva as compared with the difficulties encountered with the firmer epithelium between the network of scales seems to offer an obvious explanation of why such a concentration might occur. In mild infections the cornea is attacked and sometimes destroyed. If the infection is not treated, destruction of the eye follows, probably due to the effects of both *Epibdella* and secondary bacterial invaders. In very heavy infections the epidermis may suffer such severe injury as to cause the falling off of scales and the exposure of large areas of connective and muscular tissues with subsequent death of the fish. In one case, that of a Galapagos labroid which had escaped attention for some time, over two thousand adult worms were removed from the entire surface of the body. In the case of another similar infection several thousand eggs were found in the gill and nasal cavities. Both of these severe infections resulted in the death of the fishes.

Discussion

The life history of *Epibdella melleni* differs widely from the other definitely-known life histories of the Monogena. The life history of *E. melleni* seems to offer no homologies to the polyembryonic or pædogenetic condition found in *Gyrodactylus* (Kathariner, 1904) or to the characteristic fusion of the diporpa larvæ of *Diplozoön* (Zeller, 1872*a*). The direct development of a ciliated larva into the adult seems to be more closely related to the type of development described for *Polystomum integerrimum* (Zeller, 1872, 1876). However, the dimorphic development described for this species is probably, as pointed out by previous workers (*e.g.*, Stunkard, 1917), the result of the confusion of the life histories of two different species. It seems probable that the ciliated larva described by Zeller may develop into one of the two forms which he describes as the adult. If the development is similar to that found in *Epibdella*, it is quite probable that the ciliated larva of Zeller developed into the adult which was found on the gills of the tadpole. Also, if the ciliated larva of Zeller is shown to be the larva of the form in the urinary bladder, it is possible that a comparison of the two cases of direct development may still be drawn.

The wide differences between the life histories of *Epibdella* and *Gyrodactylus* seem to have an important bearing on the taxonomic grouping of the members of the order. Fuhrmann (1928) removed from the suborder Monopisthocotylinea Odhner the three families Protogyrodactylidæ, Gyrodactylidæ, and Calceostomidæ and created for them a new suborder, Monopisthodiscinea, of which the distinguishing characteristic was the absence of a definite posterior sucker



Text Fig. 2. Diagrams showing the possible development of the intra-ovarian seminal receptacle. A. Relationships of seminal receptacle (*sr*), oviduct (*od*), and ovary (*ov*) in some of the other species of Monogena. B. A hypothetical intermediate stage. C. The relationship as found in *Epibdella melleni*. The boundary between the seminal receptacle and the ovary was not seen as a double wall, but it is quite probable that the two walls fused during development to form one wall as could be seen in sections.

and the possession of a posterior, armed, adhesive disk. A comparison of the life histories of *Gyrodactylus* and *Epibdella*, which may be considered as characteristic members of their respective suborders, seems to offer very good embryological evidence that the separation of *Gyrodactylus* and its close relatives from the other members of the suborder Monopisthocotylinea was well warranted. Thus it seems as if the differences between the two groups are very fundamental and that they may be divided on the basis of their embryology as well as on the basis of their adult morphology.

The presence of the seminal receptacle within the ovary has not, to the knowledge of the authors, been reported previously in the order Trematoda. Inasmuch as a seminal receptacle is found as an evagination of the oviduct in other species of the Monogena, it is thought that the unusual seminal receptacle noted in *Epibdella melleni* is probably homologous with this structure in other species. Since the oviduct may be traced not only to but also inside of the ovary, it is thought that the intra-ovarian position of the seminal receptacle is brought about either by an overgrowth of the ovary around the seminal receptacle or by the invagination of the receptacle and basal portion of the oviduct within the ovary. Text figure 1 shows how such a rearrangement might have taken place. The wall around the receptacle could not be seen as a double wall as shown in the diagram, but it seems quite probable that the wall of the ovary and the wall of the receptacle might become fused during development, thus giving rise to one relatively thick wall as was seen in sections.

In view of the discrepancies between the original description of *Epibdella melleni* by MacCallum (1927) and the observations of the present authors, it is thought advisable that the description of the species be modified to read as follows:

Family Tristomidæ Taschenberg,
Subfamily Tristominæ Monticelli,
Genus *Epibdella* Blainville 1828,
Epibdella melleni MacCallum 1927.

Ectoparasitic on the eyes and epidermis and sometimes in the gill and nasal cavities of numerous marine fishes of the order Acanthopteri. Body oval, measuring 2–5 $\times$ 1.5–3.0 mm., with definite anterior suckers; skin smooth, posterior sucker with two pairs of hooks, one pair of spines, and fourteen very small (10 μ) larval hooklets, but no papillæ. Digestive tract greatly ramified. Cirrus sac pyriform, divided internally to form two saccules, the seminal vesicle and the prostatic reservoir. Two prostate glands; no vagina, uterus, nor genito-intestinal canal. Seminal receptacle within the ovary. Eggs tetrahedral, with two short hooks and one long filament.

Larva approximately 225 microns in length with three ciliated bands in the anterior, middle, and posterior regions of the body. Four large conspicuous eyes. Digestive system simple. Posterior sucker remains closed until after attachment. Development into adult is direct.

Summary

1. The description of the adult of *Epibdella melleni* MacCallum 1927 is corrected and extended, and the complete life history of the parasite is described.

2. The morphology of the larva of *E. melleni*, including the excretory pattern, is described in detail.

3. The general outline of the development of the larva into the adult is traced.

4. Susceptibility to infection with *E. melleni* has been found to be limited to certain families of the order Acanthopteri.

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EXPLANATION OF PLATES

1. Camera lucida drawing of the adult of *Epibdella melleni* showing the principal organ systems. The vitellaria fill most of the interstitial space throughout the body and are not shown in the figure. Size of specimen is 4 millimeters.

2. Sagittal section through anterior half of adult specimen.

3. Sagittal section through the posterior sucker showing the secondary margin.

4. Free-swimming larva of *E. melleni*, drawn from living material, shortly after hatching. Size of specimen, 225 μ.

5. Larva which had been isolated when ciliated and maintained under a sealed coverslip for forty-five minutes, during which time the ciliated epithelium was shed and the posterior sucker opened.

6. Young individual obtained from an infected fish. Size of specimen, 320 μ.

7. Diagram of the excretory pattern of free-swimming larva.

8. Most common type of egg of *E. melleni.* Length of body of egg, 150 μ.

9. Somewhat less common type of egg.

10. Rather unusual form of egg which differs from the others in the ratio of the lengths of the edges of the tetrahedron.

11. Abnormal egg, shape probably the result of insufficient shell material in the oötype at time of formation.

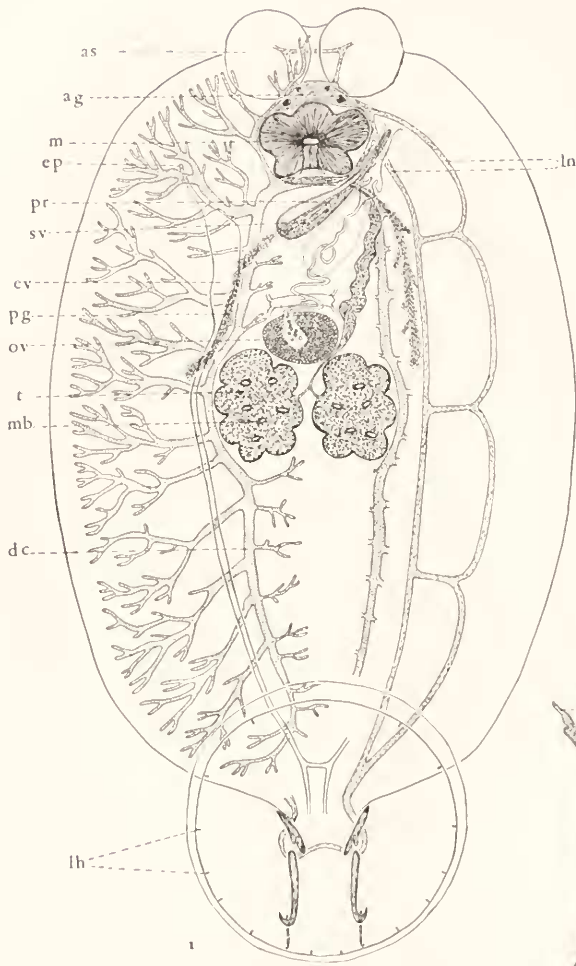
12. Filament as cast out when apparently still less shell material is available for formation of the body of the egg.

13. Larval hook. Specimen 10 μ in length. *a*, edge view; *b*, side view.

Abbreviations

ag anterior nervous ganglion
as anterior sucker
c cirrus
cs cirrus sac
dc digestive cæcum
e eye
ep excretory pore
ev excretory vesicle
gs genital sinus
lh larval hooks
ln longitudinal nerve trunk
m mouth
mb muscular band through testis
mt metraterm
od oviduct
oes œsophagus
ol oral lips
ot oötype
os oral sucker
ov ovary
pg prostate gland
pog post oral ganglion
pr prostatic reservoir
sg 'shell gland'
sgd 'shell gland' duct
sm secondary margin of posterior sucker
sr seminal receptacle
sv seminal vesicle
t testis
vd vas deferens
yd vitelline duct
yr yolk reservoir

PLATE 1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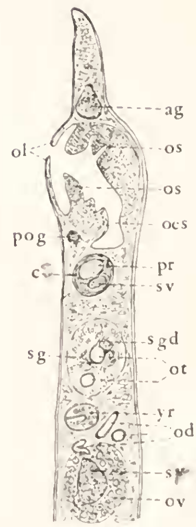
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PLATE 2